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[続葉有]

(54) Title: MOUSE HAVING HUMAN CYTOCHROME P450 TRANSFERRED THEREIN

(54) 発明の名称: ヒトチトクロームP450遺伝子導入マウス

(57) Abstract: A fragment of the 7th chromosome originating in normal human fibroblast is transferred into a mouse ES cell (embryonic stem cell) by the microcell method. By using this ES cell, a chimeric mouse, which sustains the human chromosome fragment in normal tissues and expresses human CYP3A4 gene in the liver and the small intestine, is obtained. To disrupt mouse endogenous P450 genes, a physical map concerning Cyp3a genes on the mouse 5th chromosome is formed. Based on this physical map, a vector required in gene targeting is constructed. By using this vector, a mouse, which has the human P450 genes (CYP3A family) and in which the mouse endogenous P450 genes (Cyp3a family) have been disrupted, is constructed.

(57) 要約:

ヒト正常線維芽細胞由来7番染色体の部分断片をミクロセル法によりマウスES細胞（胚性幹細胞）に導入し、このES細胞を用いて正常組織においてヒト染色体断片を保持し、薬剤の誘導によって肝臓、小腸においてヒトCYP3A4遺伝子を発現するキメラマウスを得る。また、マウス内在性P450遺伝子群を破壊するために、マウス5番染色体上のCyp3a遺伝子群に関する物理的地図を作製するとともに、その物理的地図をもとに遺伝子ターゲティングに必要なベクターを作製し、それを用いて、ヒトP450遺伝子（CYP3Aファミリー）を有し、さらにマウス内在性のP450遺伝子（Cyp3aファミリー）が破壊されたマウスを作製する。

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MOUSE HAVING HUMAN CYTOCHROME P450 TRANSFERRED THEREIN

Description of correspondent: **EP1206906**

TECHNICAL FIELD

[0001] The present invention has an object of providing a nonhuman animal that shows human type drug metabolism, and it relates to a nonhuman animal having introduced therein a human chromosome fragment containing cytochrome P450 genes and a method of preparing the same.

BACKGROUND

[0002] In vivo metabolism of drugs is mainly performed by cytochrome P450 (hereinafter referred to as "P450") existing in the liver. P450 forms a super family consisting of many genes. P450s having a homology on an amino acid sequence in excess of 40% are grouped into the same family, and those having an amino acid sequence homology of 55% or more are classified into a subfamily (Nelson et al., Pharmacogenetics, 6:1, 1996). Human P450 and rat P450 belonging to the same subfamily show a difference in their properties upon comparison and sometimes a difference in a substance serving as a substrate or in a metabolite may be observed. For this reason, information on the metabolism of a certain drug in rat cannot be applied as it is to humans, and hence development of a test system for accurately anticipating the metabolism of a drug in human is desired (Funae et al., Bioscience and Industry, 55:81, 1997).

[0003] Basically, when examining the metabolism of drugs in human, it is the best way to use human liver microsome. However, it is difficult to obtain the human liver microsome. On the other hand, it has been becoming possible to relatively easily prepare human enzymes by genetic engineering techniques by which enzymes having the same standard can be stably supplied, and thus utilization of them may be contemplated (Kamataki, Reports of Anhyo Center Laboratory, Incorporated Foundation, 7:27, 1997).

[0004] On the other hand, in vitro systems have been constructed in order to examine influence of drugs metabolized and activated by P450 on living organisms. In this approach, liver microsome and a drug are added to cell culture medium and influence of a metabolite having been subjected to extracellular metabolism on the above-mentioned cells is observed. In this case, the activated substance is adsorbed on a cell membrane and only a portion thereof can reach inside of the cells. Therefore, there is a possibility that the influence of the metabolite on the cells cannot be accurately grasped. In a case where cells themselves express P450, a drug that has invaded into the cells without being adsorbed on the cell membrane may be considered to be activated in the cells, so that it is considered that influences in which the metabolite gives including toxicity can be properly reproduced. From this point of view, it has been considered that it is desirable to use cells in which human P450 gene has been introduced for an evaluation of toxicity of metabolites (Kamataki et al., Toxicology Letters, 82-83:879, 1995).

[0005] However, under the present circumstances, the above-mentioned respective methods using an in vitro expression system have certain problems. Although the expression system using yeast having introduced therein human P450 (for example, Kovaleva et al., Biochem. Biophys. Res. Commun., 221:129, 1996) has advantages that a certain amount of expression of P450 is obtained and expression can occur without modifying P450 cDNA, the system has a problem that the system itself contains P450 of yeast itself. Although the expression system using Escherichia coli (for example, Gillam et al., Arch. Biochem. Biophys., 305:123, 1993) is easy to handle and a large amount of enzyme can be obtained, it is necessary to modify N-terminal amino acid of P450 to be expressed in order to perform expression stably. Also there are problems that a possibility that this modification will influence enzyme activity is suggested and that Escherichia coli has no reductase necessary for exhibiting P450 activity so that a reductase must be additionally added and the like. The system using insect cells and Baculovirus (for example, Asseffa et al., Arch. Biochem. Biophys., 274:481, 1989) gives a large expression amount and needs no modification of the N-terminal amino acid; however, a certain skill is required for a manipulation for the expression. The system using a human hepatoma-derived Hep G2 cell and a vaccinia virus (for example, Shou et al., Mol. Carcinog., 10:159, 1994) and the system using human B lymphocyte may use human cells and P450 is considered to be expressed in a state closer to the in vivo environment. However, in the case where use of the vaccinia virus is involved or the case where Hep G2 cell microsome is used, consideration must be given to safety (Funae et al., Bioscience and Industry, 55 : 81, 1997).